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Ecophysiological Response of *Capsicum annuum* L. Exposed to Simulated Acid Rain

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Abstract

Pot experiments were carried out to evaluate the effects of simulated acid rain (pH 5.6 (control), 5.0, 4.0, 3.0, and 2.0) exposures on *Capsicum annuum* L. All pH levels with the exception of the control adversely affected all growth parameters (plant height, number of leaves, fresh and dry weight of leaves, root, stem and fruits, stem girth) of the plant. Leaf area and fruit production were also impacted. Nutritional analysis of the leaves revealed a decline with increasing acidity levels. Simulated acid rain induced morphological changes such chlorosis, necrosis, early leaf senescence, leaf abscission and death. The reduction in growth parameters were concentration dependent. The most negative effects of simulated acid rain on the plant growth parameters and nutrient content analysis were observed in pH 2.0 treatment.

Key words: Acid rain, *Capsicum annuum*, growth parameters, morphological changes, pH.

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Introduction

Acid rain has been defined as the wet deposition of pollutants such as oxides of sulphur and nitrogen contained in power plant emission, factory smoke and car exhaust, which react with the moisture in the atmosphere (Kita *et al*., 2004). Rain that presents a concentration of H^+ ions greater than 2.5 μeq^{-1} and pH values lower than 5.6 is considered as being acidic. It has been recognized that herbaceous plants are more sensitive to direct injury by acid rain than woody plants (Heck *et al.*, 1986). The adverse effects of acid rain include chlorosis, necrosis, early senescence and stunting (Evans *et al.*, 1997). The harmful effects of acid rain have been reported on some crop plants such as *Triticum aestivum*, *Solanum lycopersicum*, *Glycine max*, *Lens culinaris* and *Coriandrum sativum* (Singh, 1989; Kausar*et al.,* 2006; Kazim, 2007). Simulated acid rain has also been known to effect a reduction in plant growth and yield of *Zea mays* and *Solanum lycopersicum* (Banwart *et al.,* 1988; Shripal *et al.,* 2000; Dursun *et al.,* 2002).

Bell pepper (*Capsicum annuum* L.) belongs to the family Solanaceae. They are tropical plants and thus suited to hot and humid conditions. *Capsicum* fruits can be eaten raw or cooked. The fruit of most species of *Capsicum* is known to contain capsaicin, a lilophilic chemical that can produce strong burning sensation in the mouth of the unaccustomed eater. Most mammals find this unpleasant, whereas birds are unaffected (Mason *et al*., 1991; Norman *et al*., 1998). Chili peppers are of great importance in Native American medicine, and capsaicin is used in modern medicine mainly in topical medications as a circulatory stimulant and analgesic. In more recent times, an aerosol extract of capsaicin, usually known as capsicum or pepper spray, has become widely used by police forces as a non-lethal means of incapacitating individuals and in a more widely dispersed form for riot control and personal defense. Cayenne is also very high in vitamin C so it acts as a preventative against respiratory infections and can help strengthen the immune system. It has also been proposed that the capsaicinoids might be useful in fighting cancer. The reports available for rainfall acidity on crop plants are mainly derived from studies conducted in the temperate regions, with little or no research findings in the tropics. Therefore, the objective of the present study was to examine the growth, nutrient relations and yield of *Capsicum annuum* exposed to simulated acid rain

Materials and Methods

Planting Procedure: The experiment was conducted in the screen house of the department

of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Seeds were planted directly into experimental pots containing loamy soil. Three viable seeds of *Capsicum annuum* were sown into the soil at a depth of 3cm. The seedlings were watered and grown for three (3) weeks after which thinning was carried out to reduce the plant to one (1) per pot. The plants were grown for six weeks before treatments commenced. Each pH treatment had four replicates and the experiment was arranged in a completely randomised design (CRD). Simulated acid rain was sprayed to the planted *Capsicum annuum* plants according to their pH values of 2.0, 3.0, 4.0, 5.0, and 5.6 (control). The solutions were applied using a medium size pressurized sprayer on the plants. The plants were sprayed with simulated acid rain for seven weeks before the experiment was terminated.

Preparation of Simulated Acid Rain: The acids used consist of a mixture of concentrated Tetraoxosulphate (VI) acid (H_2SO_4) and concentrated nitric acid (HNO_3) in 3:1 ratio. The acidic solution was then diluted with distilled water and a Digital Hanna pH meter was used to get the desired pH levels (2.0, 3.0. 4.0, 5.0 and 5.6).

Data collection: Several parameters were used in the assessment of the growth and productivity of the plant. The height of shoot of the plants from the soil level to the top of the plant stems were measured using a meter rule. The measurements were taken weekly from the week acid spraying commenced till the day of harvest. The number of leaves, fruits and flowers on the plant was determined by counting. Leaf area was determined by the proportional method of weighing a cut-out of traced area of the leaves on graph paper with standard paper of known weight to area ratio. The stem girth was determined with the aid of a vernier caliper. The Chlorophyll content index of the leaves was measured using the Apogee chlorophyll content meter CCM-200 plus. The number of flowers and fruits on the plant were counted at an interval of seven (7) days from the period of initiation (week 4) to the time of harvest (week 7).

Elemental analysis was carried out at the Central Analytical Laboratory, Nigerian Institute for Oil Palm Research, NIFOR to determine the level of N, P, K, Ca, Mg, Na, and Fe in the leaves of *Capsicum annuum.* Ca, Mg, and Fe, content were determined with Atomic Absorption spectrometer, Bulk Scientific VGP 210. Na and K were determined using flame photometer while Phosphorus and Nitrogen were determined using colorimetric method.The fresh and dry weights were determined after seven weeks of treatment following the method of Hunt (1990).

Statistical Analysis: Data obtained were subjected to analysis using the Statistical Package for Social Sciences, Version 20.0. Treatment means were separated using the Duncan's multiple range test.

Results

The results on the plant height, number of leaves, number of flowers and number of fruits are presented in Table 1. There was a significant decline ($P<0.05$) in all parameters with increasing acidity levels.

Table 1 Effect of simulated acid rain (SAR) on plant height (cm), number of leaves, number of flowers, and number of fruits of *Capsicum annuum*, 7 weeks after treatment

pH of SAR	Plant height (cm)	Number of leaves	Number of flowers	Number of fruits
5.6 (control)	24.50 ± 0.29^{b}	41.75 ± 1.03^{e}	10.00 ± 2.04^{b}	6.50± 1.50^{c}
5.0	21.75 ± 0.95^{b}	31.75 ± 1.38^{d}	6.75 ± 0.85^{b}	3.25± 0.95^{b}
4.0	20.25 ± 0.95^{a}	22.50 ± 0.50^{c}	2.00 ± 0.91^{a}	0.7+ 0.48^{ab}
3.0	15.75 ± 2.14^{a}	19.25 ± 0.63^{b}	1.50 ± 0.50^{a}	0.75+ 0.25^{ab}
2.0	15.25 ± 1.31^{a}	14.00 ± 1.22^{a}	0.00 ± 0.00^{a}	0.00± 0.00^{a}

KEY; Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter are not significantly different at ($P>0.05$) from each other using Duncan Multiple Range Test.

The results of exposure of *C. annuum* to SAR (Stimulated acid rain) on its stem girth, leaf area and chlorophyll content is presented in Table 2. There was a significant reduction in the stem girth, the leaf area, and the chlorophyll content with decreasing pH levels. There was a significant reduction in plant growth parameters with increasing acidity levels. Control plants had the highest value for stem girth, leaf area and chlorophyll content index.

The results on the fresh and dry weight of *Capsicum annuum* is presented in Table 3. There was a significant reduction in the fresh and dry weight with decreasing pH level. The control plant

had the highest fresh and dry weight compared to other simulated acid rain treatments.

Table 2: Effect of simulated acid rain (SAR) on the stem girth (cm), leaf area (cm^2), and chlorophyll content index (cci) of *Capsicum annuum*, 7 weeks after treatment

pH of SAR	Stem girth (cm)	Leaf area (cm^2)	cci
5.6 (control)	0.75+ 0.29[b]	15.23+ 3.1[b]	24.40 + 5.29[a]
5.0	0.55 + 0.29[a]	8.50+ 2.7[a]	16.12+ 3.78[a]
4.0	0.50+ 0.41[a]	7.12.+ 2.23[a]	20.70+ 2.35[a]
3.0	0.46 + 0.97[a]	1.97+ 1.17[a]	16.75+ 1.30[a]
2.0	0.39+ 0.89[a]	3.4 + 2.52[a]	15.60+ 2.92[a]

KEY: Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter are not significantly different at (*P>0.05*) from each other using Duncan multiple range test.

Table 3. Effect of simulated acid rain (SAR) on fresh weight and dry weight of *Capsicum annuum*, 7 weeks after treatment

pH of SAR	Fresh weight (g)	Dry weight (g)
5.6 (control)	28.79+ 8.29[b]	13.48+ 3.86[b]
5.0	9.23+ 1.62[a]	3.47+ 0.78[a]
4.0	7.66+ 2.99[a]	2.67+ 1.19[a]
3.0	2.48+ 1.17[a]	1.00+ 0.48[a]
2.0	3.12+ 1.33[a]	1.47+ 0.97[a]

KEY: Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter are not significantly different at (*P>0.05*) from each other using New Duncan Multiple Range Test.

The results of nutrient accumulated in the leaves of *Capsicum annuum* is presented in Table 4. Nutrients accumulated in the leaves of *C. annuum* for control plants had the highest value compared to those exposed to simulated acid rain.

Table 4: Nutrient accumulated in the leaves of *Capsicum annuum* treated with simulated acid rain

Taxon	pH of SAR	N(%)	P (%)	K (%)	Ca(%)	Mg(%)	Na(%)	Fe(ppm)
Capsicum annuum	5.6 (control)	4.23	0.062	1.63	2.48	1.60	0.16	0.80
	5.0	2.63	0.044	1.02	2.40	1.22	0.07	0.60
	4.0	2.76	0.034	1.17	2.22	0.88	0.09	0.40
	3.0	1.95	0.051	1.19	2.16	0.34	0.20	0.35
	2.0	2.85	0.037	1.10	1.74	0.53	0.10	0.12

Morphological changes were observed throughout the period of the experiment. Table 5 showed the effects of simulated acid rain on the morphology of *C. annuum*.

Table 5: Symptoms of different levels of simulated acid rain on overall physiology of *Capsicum annuum*

pH of SAR	Observed effects/ symptoms
5.6 (control)	luxuriant growth
5.0	Older leaves show signs of chlorosis but had good growth
4.0	Plant growth was stunted, but plant had good growth
3.0	Plant growth was stunted and necrotic lesions were visible on the leaves
2.0	The leaves were chlorotic. Plant growth was stunted.

Discussion

Acid rain is known to impact all living and non-living sub-stances. In the present study five pH levels (5.6, 5.0, 4.0, 3.0, 2.0) of simulated acid rain were applied on *C.annuum.* Simulated acid rain treatment deposited on leaves affect mainly the epidermal cells causing erosion of the cuticle and altering the leaf permeability (Evans, 1984). Symptoms of plants polluted with simulated acid rain included; chlorosis, necrosis, stunted growth, lesion, suppression of leaf production, leaf curling, withering of leaves, leaf abscission and even death of plants. Silva *et al*. (2006) reported that plants exposed to low pH rain (pH 3.0) are generally retarded with leaf chlorosis, necrotic spot coupled with dehydration of the plants. In *C. annuum*, chlorotic symptoms were observed in all acid rain treated plants four (4) weeks after treatment

commenced. The acute symptom was observed at 2.0 pH level. pH levels of 2.0, 3.0, 4.0 adversely affected growth of *C. annuum*. The reduced plant growth might have been caused by the injuries, reduced photosynthesis and other physiological disorders. The reduction in plant growth have also been observed in Wheat, Sunflower and Coriander (Kausar *et al.,* 2006; Mustabeen, 2006; Kazim, 2007). Waldron (1978) stated that the pH level is responsible for reduction in growth. It has been reported that plants sensitive to acid rain can show changes in their morphology, anatomy, physiology and biochemistry (Neufeld *et al*., 1985). All the plant growth parameters studied such as plant height, number of leaves, number of flowers, number of fruits, fresh weight and dry weight decreased significantly at all pH levels with respect to the control treatment. The highest reduction was observed at pH 2.0 level (Table 1 and 3). The adverse effects of simulated acid rain on plant growth parameters on several crops were also reported by Banwart *et al*. (1990), Evans *et al*.(1997) and Halman *et al.* (2008). Photosynthetic pigments were also inhibited in response to acidity levels. Stem girth and leaf area were reduced by simulated acid rain treatment relative to the control at pH 2.0 and pH 3.0 (Table 2).

Walker *et al*. (2001) stated that the availability of nitrogen in the soil is known to directly affect the growth of plants. Nitrogen is an essential macronutrient needed by all plants to thrive. It is an important component of many structural, genetic and metabolic compounds in plant cells. It is also one of the basic components of chlorophyll, which is essential for photosynthesis. The significant reduction in the amount of nitrogen in plants exposed to simulated acid rain could be partly responsible for their poor growth performance (Veukova *et al*., 1999). The observed stunted growth for plants treated with SAR pH2.0 was similar to an earlier study by Evans (1984). Iron is needed to produce chlorophyll. Its deficiency resulted in chlorosis, which compromised the photosynthetic capability of the plant. Expectedly, in all elemental analysis carried out, the control plants had the maximal value of nutrients accumulated in the leaves.

Conclusion

It is evident from this study that simulated acid rain with increased acidity levels had negative effects on the growth and yield of *C. annuum*. The exposed plants showed reduced growth with increasing acidity due to a reduction of photosynthesis as a result of chlorosis, necrosis and leaf abscission. Exposure to simulated acid rain at pH 4.0 and below markedly suppressed growth characteristics and yield of *Capsicum annuum*.

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